PATENT
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#21

Applicants	:	Phillipe JEANNIN
Appln. Serial No.	:	08/863,692
Filed	:	May 27, 1997
For	:	**INSECTICIDAL COMBINATION TO CONTROL MAMMAL FLEAS, IN PARTICULAR FLEAS ON CATS AND DOGS**
Examiner	:	S. Clardy
GAU	:	1616

Duplicate copy (unsigned)

745 Fifth Avenue
New York, New York 10151

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Dr. Alan A. Marchiondo, declares and says that:

1. I am currently employed by Merial LTD, the assignee of the above-captioned application, where I am a research scientist. My responsibilities include conducting pharmaceutical and biological evaluations (basic, preclinical or clinical), and target animal safety trials for development research and registration of animal health products in regional and global markets. I am responsible for supervising the maintenance and propagation of ecto— and endoparasite colonies/cultures for *in vitro* and *in vivo* efficacy trials. I have read and am familiar with the application.

2. My education, training and experience are as follows:

As evidenced from my curriculum vitae copy attached hereto, I have extensive training and experience in the field of parasitology. Accordingly, I am considered by my peers to be an expert in the field to which the application pertains.

3. I am making this Declaration in response to comments raised in the Office Action dated March 24, 1999, including the remarks which criticized the fact that there was no comparison of the inventive composition comprising compound (A) and compound (B) against a composition comprising either compound (A) and compound (B) alone. In response to those remarks the following experiments were conducted:

4. Under my direct supervision and control, tests have now been conducted which compare the inventive long-lasting compositions comprising of fipronil and (S)-methoprene with each of the two components alone.

5. The results of these tests, as reported in the Tables, demonstrate that the inventive compositions possess surprising and unexpected synergistic ovicidal activity in contrast to the results which might have been expected by an evaluation of the individual components according to the Additive Evaluation Method.

6. Specifically, under my supervisions and control, the following *in vitro* tests, as reported in Tables 1 to 6, demonstrate that surprisingly unexpected and synergistic results are observed by the inventive compositions.

A. Egg Eclosion Test

This test determines the emergence of insect larva from an egg. Each test solution, described below, is applied to a filter paper disc. Approximately twenty-five flea eggs, which are less than twenty-four hours old, are applied to each of the discs. The discs are then incubated at an adequate temperature (approximately 23-30° C) and relative humidity (approximately 70-90% RH) to support cat flea (*Ctenocephalides felis*) development for approximately 72 hours following deposition of the flea eggs onto the filter paper discs. The

geometric mean of the flea larvae that hatched from four replicates of 25 flea eggs each at each concentration tested is presented in Table 1.

Table 1: Geometric Mean of Hatched Flea Larvae

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
CONTROL	18.25	23.25	18.00
10	20.00	17.00	ND
50	18.25	3.25	ND
10 + 10	ND	ND	10.50
50 + 50	ND	ND	1.25
10 + 50	ND	ND	2.75
50 + 10	ND	ND	2.00

ND = NOT DETERMINED

In Table 1, the amount of hatched larvae when fipronil is used alone is very similar to the control at both concentrations. Thus, one would conclude that fipronil, when used alone at these concentrations, is either inactive or slightly active and (S)-methoprene, while more active than fipronil alone at 10 ppm, killed only approximately 25% of the flea eggs (see Table 2). In contrast thereto, significant activity is observed for all the compositions containing both fipronil and (S)-methoprene.

Table 2 presents the data from Table 1 as the percent inhibition of insect larva from the egg.

Table 2: Percent Inhibition of Egg Eclosion

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
10	0	26.9	ND
50	5.2	86.0	ND
10 + 10	ND	ND	41.9 (0 + 26.9)
50 + 50	ND	ND	93.1 (5.2 + 86.0)
10 + 50	ND	ND	84.7 (0 + 86)
50 + 10	ND	ND	88.9 (5.2 + 26.9)

ND = NOT DETERMINED

The data presented in parenthesis is the percent inhibition of the active substances by the Additive Evaluation Method.

As can be seen, at 10 ppm, (S)-methoprene is about 27 times more active than fipronil, which is inactive at that amount. At 50 ppm (S)-methoprene is about 16.5 times more effective than fipronil, which only inhibits the larval emergence by 5.2%. Moreover, when these two agents are combined, clearly superior activity is obtained when the composition contains 10 ppm of (S)-methoprene is present. (see e.g. 10:10 mixture or 50:10 mixture). Such a result clearly suggests synergism at lower concentrations.

B. Larval Pupation Results

This test determines the effect an active agent has on the pupation of the larvae. After the number of hatched flea larvae have been counted after approximately 72 hours following flea egg deposition on the discs, the discs are further incubated at approximately 23-30° C and approximately 70-90% RH for approximately 14-20 days following deposition of the flea eggs onto the filter paper discs. After this period, the pupae are then separated into a container, counted and the number recorded.

The geometric mean of the pupae after 20 days is reported in Table 3.

Table 3; Geometric Mean of the Pupae – 20 Days

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
CONTROL	9.1	8.2	7.7
10	1.2	0	ND
50	0.7	0	ND
10 + 10	ND	ND	0
50 + 50	ND	ND	0
10 + 50	ND	ND	0
50 + 10	ND	ND	0

ND = NOT DETERMINED

The geometric mean number of pupae was comparable between the three control groups (range of 7.7-9.1). However, only a few flea pupae developed at the fipronil concentrations of 10 and 50 ppm as evidenced by geometric means of 1.2 and 0.7 pupae,

respectively. Likewise, no pupae develop form any of the discs treated with (S)-methoprene alone or in combination with fipronil, thus demonstrating significant larvicidal activity.

The percent of larvicidal activity based upon pupae formation is reported on Table 4.

Table 4: Percent Inhibition of Pupae Formulation

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
10	86.8	100	ND
50	92.3	100	ND
10 + 10	ND	ND	100
50 + 50	ND	ND	100
10 + 50	ND	ND	100
50 + 10	ND	ND	100

ND = NOT DETERMINED

Whereas fipronil is either inactive or slightly active against flea egg eclosion at the concentrations tested, fipronil exhibited a strong inhibitory effect against the larval stages of the flea, as evidenced by percent inhibition of pupae formations of 86.8 and 92.3% at 10 and 50 ppm, respectively. All samples containing (S)-methoprene alone or in combination exhibited 100% inhibition of pupa formation. From these data, I concluded that the inventive compositions are not demonstrating their synergistic effect at the stages where the larvae pupate due to the high level of larvicidal activity demonstrated by fipronil and (S)-methoprene alone at the concentrations evaluated.

C. This test determines the effect an active agent has on the emergence of adult fleas from pupae. After the pupae have been determined from the larvicidal test, the separated pupae are returned to the incubator. On approximately Days 29-33 following deposition of flea eggs onto the discs, the pupae are agitated twice on two separate days by vigorous shaking and exposed to CO_2 in order to stimulate adult flea emergence. On day 35 following egg deposition, the pupae are again agitated and exposed to CO_2 and frozen at approximately 0° C. Once any further development and emergence has been stopped by freezing the specimens, the number of emerged adult fleas are counted and recorded. Pupae that have

not released adult fleas were dissected. Encased adults were examined and assessed for normal development. Normal encased adults were recorded with the number of emerged adult fleas.

The geometric mean of the adult flea emergence is reported in Table 5.

Table 5: Geometric Mean of Adult Flea Emergency Plus Unemerged Adult Flea

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
CONTROL	6.8	6.4	6.5
10	1.1	0	ND
50	0.6	0	ND
10 + 10	ND	ND	0
50 + 50	ND	ND	0
10 + 50	ND	ND	0
50 + 10	ND	ND	0

ND = NOT DETERMINED

The geometric mean number of adult fleas was similar in the three control groups ranging from 6.4-6.8 emerged adult cat fleas. In the fipronil group, the number of emerged adult fleas was very similar to the number of pupae indicating no effect of fipronil on adult emergence at the concentrations evaluated. Likewise, no adult cat fleas emerged form any discs treated with (S)-methoprene or the invention compositions because no pupae were formed.

The percent inhibition of adult flea development and emergence is reported in Table 6.

Table 6: The Percent Inhibition of Adult Flea Development and Emergence

CONC. (PPM)	FIPRONIL	METHOPRENE	COMBO
10	83.8	100	ND
50	91.2	100	ND
10 + 10	ND	ND	100
50 + 50	ND	ND	100
10 + 50	ND	ND	100
50 + 10	ND	ND	100

ND = NOT DETERMINED

From these data I conclude that the invention compositions do not demonstrate a synergistic effect at the stage where the adult fleas emerges from or develops within the pupal case because of the significant levels of initial ovicidal and subsequent larvicidal activities.

7. As is apparent from the reported results, these experiments demonstrate that surprisingly unexpected results are obtained at the egg hatching stage when relatively low concentrations of (S)-methoprene are combined with fipronil. The data demonstrate that the inventive combinations possess superior and synergistic activity at the stage the larvae hatches from the flea egg. As the prior art relied upon in the rejection does not suggest this result, it is clearly unexpected. Moreover, in view of the unexpected results obtained from (S)-methoprene, one could conclude that such results would also be obtained with other compounds which exert their activity mimetics of the juvenile hormone.

8. Under my supervision and control the following *in vivo* tests were conducted upon cats and dogs in order to demonstrate the surprisingly unexpected results. Specifically, these results demonstrate that the inventive combination inhibited larvae hatching from the eggs for a surprisingly long duration when the inventive combination of active agents applied to cats and dogs.

A. Ovicidal Inhibition Test in *Ctenocephalides Felis*

This test determines the effect that a flea composition has on the inhibition of larvae hatching from flea eggs and on the inhibition of adult cat flea emergence when the composition is applied to the skin of cats infested with newly emerged adult cat fleas (*Ctenocephalides felis*). Thirty-two (16 male and 16 female) domestic shorthaired cats approximately 6-12 months old and weighing 2.35-5.66 kg were selected and housed in individual cages. On Day –12 each cat was infested with approximately 200 adult cat fleas. On Day –11 the cats were combed to remove and count the fleas and they were re-infested with approximately 200 adult fleas. At approximately 72 hours post-infestation, a procedure for collection of flea eggs was begun. Eggs were collected over approximately a 24-hour period. On Day –7 two aliquots of approximately 100 eggs each were formed from the eggs collected from each animal. One of these aliquots was

incubated at approximately 23-30° C and 70-90% RH for approximately 72 hours to determine larval hatch. The other aliquot was incubated under the same conditions for 35 days to determine the number of adult fleas that developed. Eight replicates of four animals were formed based on body weight within sex. One cat in each replicate was randomly allocated to each of four treatment groups: 1) untreated control; 2) fipronil 10% w/v solution; 3) (S)-methoprene 12% w/v solution; and 4) fipronil 10% w/v and (S)-methoprene 12% w/v combination solution. Treatments of the flea compositions were applied once topically on Day 0 at the rate of 0.5 ml/cat. On days 1, 22, 29, 26, 43, 50 and 57 each cat was infested with approximately 200 adult fleas. Eggs were collected over approximately a 24-hour period beginning three days after infestation. One aliquot of up to approximately 100 eggs, if available, from each animal at each infestation time was incubated for three days to determine larval hatch and the other aliquot incubated for 35 days to determine the number of adults that developed. The results of this trial are reported in Table 7 (Cat Dose Confirmation Trial – Percentage of Larvae That Hatched) and Table 8 (Cat Dose Confirmation Trial – Percentage of Adult Fleas That Develop).

TABLE 7
Methoprene Dose Trial in Cats Percentage [A] Larvae that Hatch

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 12 % w/v	Fipronil (10% w/v) + Methoprene (12% w/v)
Pretreatment	34.8	28.3	36.5	36.9
Day 1	50.6	-	0	-
% Reduction		-	100	-
Day 22	39.8	-	0	-
% Reduction		-	100	-
Day 29	42.6	0 [C]	0.3	0 [D]
% Reduction		100	99.4	100
Day 36	34.6	42.5 [E]	5.2	1.4 [F]
% Reduction		0	85.0	95.9
Day 43	55.9	58.6	4.6	3.6
% Reduction		0	91.7	93.6
Day 50	39.2	54.9	18.9	8.7
% Reduction		0	51.6	77.9
Day 57	53.8	46.0	50.4	27.3
% Reduction		14.4	6.3	49.2

[A] Retransformed mean of radians; based on the transformation are sine √(number of adults/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 35 days.
[C] One animal with a few eggs; no adults developed.
[D] Three animals with eggs; no adults developed.
[E] Five animals with eggs incubated.
[F] Six animals with eggs incubated

TABLE 8
Methoprene Dose Confirmation Trial in Cats Percentage [A] of Adults that Develop

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 12 % w/v	Fipronil (10% w/v) + Methoprene (12% w/v)
Pretreatment	34.8	28.3	36.5	36.9
Day 1	50.6	-	0	-
% Reduction		-	**100**	-
Day 22	39.8	-	0	-
% Reduction		-	**100**	-
Day 29	42.6	0 [C]	0.3	0 [D]
% Reduction		**100**	**99.4**	**100**
Day 36	34.6	42.5 [E]	5.2	1.4 [F]
% Reduction		**0**	**85.0**	**95.9**
Day 43	55.9	58.6	4.6	3.6
% Reduction		**0**	**91.7**	**93.6**
Day 50	39.2	54.9	18.9	8.7
% Reduction		**0**	**51.6**	**77.9**
Day 57	53.8	46.0	50.4	27.3
% Reduction		**14.4**	**6.3**	**49.2**

[A] Retransformed mean of radians; based on the transformation are sine √(number of adults/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 35 days.
[C] One animal with a few eggs; no adults developed.
[D] Three animals with eggs; no adults developed.
[E] Five animals with eggs incubated.
[F] Six animals with eggs incubated

The results of these trials are depicted in Fig. 1 and Fig. 2 respectively.

As is apparent from the data, the inventive compositions comprising fipronil and (S)-methoprene are surprisingly more ovicidally active for an extended time than composition comprising just fipronil or (S)-methoprene alone.

B. Ovicidal Inhibition Tests in Dogs

This test determines the effect that a flea composition has on the inhibition of larvae hatching from fleas eggs and on the inhibition of adult cat fleas emergence when the composition is applied to the skin of dogs infested with newly emerged adult cat fleas (*Ctenocephalides felis*). Thirty-six (8 female and 24 male) Beagle dogs approximately 12.7-65.9

months old and weighing 10.2-19.8 kg were selected and housed in individual runs. On Day –12 each dog was infested with approximately 200 adult cat fleas. One Day –11 the dogs were combed to remove and count the fleas and they were re-infested with approximately 200 adult fleas. At approximately 72 hours post-infestation, a procedure for collection of flea eggs was begun. Eggs were collected over approximately a 24-hour period. On Day –7 two aliquots of approximately 100 eggs each were formed from the eggs collected form each animal. One of these aliquots was incubated at approximately 23-30° C and 70-90% RH for approximately 72 hours to determine larval hatch. The other aliquot was incubated under the same conditions for 35 days to determine the number of adult fleas that developed. Eight replicates of four animals were formed (four dogs were dropped from the trial) based on body weight within sex. One dog in each replicate was randomly allocated to each of four treatment groups 1) untreated control; 2) fipronil 10% w/v solution; 3) (S)-methoprene 9% w/v solution; and 4) fipronil 10% w/v and (S)-methoprene 9% w/v combination solution. Treatments of the flea composition were applied once topically on Day 0 at the rate 0.067 ml/kg body weight. On days 1, 22 and weekly to Day 85 each dog was infested with approximately 200 adult fleas. Eggs were collected over approximately 100 eggs, if available, from each animal at each infestation time was incubated for three days to determine larval hatch and the other aliquot incubated for 35 days to determine the number of adults that developed. The results of this trial are reported in Table 9 (Dog Dose Confirmation Trial – Percentage of Larvae That Hatch) and Table 10 (Dog Dose Confirmation Trial – Percentage of Adult Fleas That Develop).

TABLE 9
Methoprene Dose Trial in Dogs Percentage [A] of Larvae that Hatch

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 9 % w/v	Fipronil (10% w/v) + Methoprene (9% w/v)
Pretreatment	74.9	71.9	71.0	68.4
Day 1	77.6	-	0	-
% Reduction		-	**100**	-
Day 22	76.4	-	0.1	-
% Reduction		-	**99.8**	-
Day 29	75.0	-	0.8	-
% Reduction		-	**98.9**	-
Day 36	63.7	22.2*	2.7	-
% Reduction		**65.1***	**95.8**	-
Day 43	78.8	51.6**	5.3	1.8***
% Reduction		**34.5****	**93.9**	**97.8*****
Day 50	75.1	49.4	6.0	6.8
% Reduction		**34.3**	**92.0**	**90.9**
Day 57	76.8	56.2	29.7	1.4
% Reduction		**26.8**	**61.3**	**98.2**
Day 64	78.0	59.2	30.0	8.5
% Reduction		**24.2**	**61.5**	**89.1**
Day 71	75.3	62.7	30.6	8.1
% Reduction		**16.7**	**59.3**	**89.3**
Day 78	85.3	49.4	30.6	12.4
% Reduction		**42.1**	**64.1**	**85.5**
Day 85	76.1	61.8	50.4	26.9
% Reduction		**18.8**	**33.7**	**64.6**

[A] Retransformed mean of radians; based on the transformation are sine √(number of larvae/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 72 hours.
* One animal with 36 eggs incubated; 8 larvae hatched (22.2%).
** Six animals with eggs incubated.
*** Three animals with eggs incubated.

TABLE 10
Methoprene Confirmation Trial in Dogs Percentage [A] of Adults that Develop

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 9 % w/v	Fipronil (10% w/v) + Methoprene (9% w/v)
Pretreatment	55.3	58.0	57.5	56.1
Day 1	56.5	-	0	-
% Reduction		-	100	-
Day 22	57.4	-	0.1	-
% Reduction		-	99.8	-
Day 29	55.5	-	0.8	-
% Reduction		-	98.5	-
Day 36	56.9	-*	0.5	-
% Reduction		-*	99.2	-
Day 43	59.2	20.5**	2.5	0.7***
% Reduction		65.4**	95.8	98.8***
Day 50	53.5	23.2	4.7	2.2
% Reduction		56.7	91.3	95.9
Day 57	57.4	38.8	13.8	0.3
% Reduction		32.5	75.9	99.4
Day 64	55.5	39.2	18.9	3.8
% Reduction		29.5	66.1	93.1
Day 71	53.5	45.7	13.9	2.2
% Reduction		14.6	74.0	95.9
Day 78				
% Reduction				
Day 85				
% Reduction				

[A] Retransformed mean of radians; based on the transformation arc sine √(number of adults/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 35 days.
* One animal with 35 eggs incubated; 4 adults developed (11.4%).
** Six animals with eggs incubated.
*** Three animals with eggs incubated.

The results of these two trials are depicted in Fig. 3 and Fig. 4 respectively.

As is apparent from the data, the inventive composition comprising fipronil and methoprene are surprisingly more ovicidally active for an extended period of time than compositions comprising just fipronil or (S)-methoprene alone.

9. Based upon the data presented above, I conclude that the inventive composition comprising a combination of fipronil and (S)-methoprene exhibit surprisingly superior ovicidal activity in inhibiting larvae from hatching and for extended periods of time. As one would not expect this in view of the state of the art this activity is unexpected. Moreover, from this observation one would expect that the inventive compositions would have economic

importance in the marketplace in view of their long duration. Further, in view of the fact that insect growth possesses similar activities, one could also conclude that such results would be obtained with other compounds which exert their activity by mimicking the juvenile hormone.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____, 1999 By: _____
Alan Marchiondo, Ph.D.

Fig. 1

- % Reduction in Proportion of Larvae that Hatched by Treatment and Day of Flea Challenge (Eight Cats per Treatment)

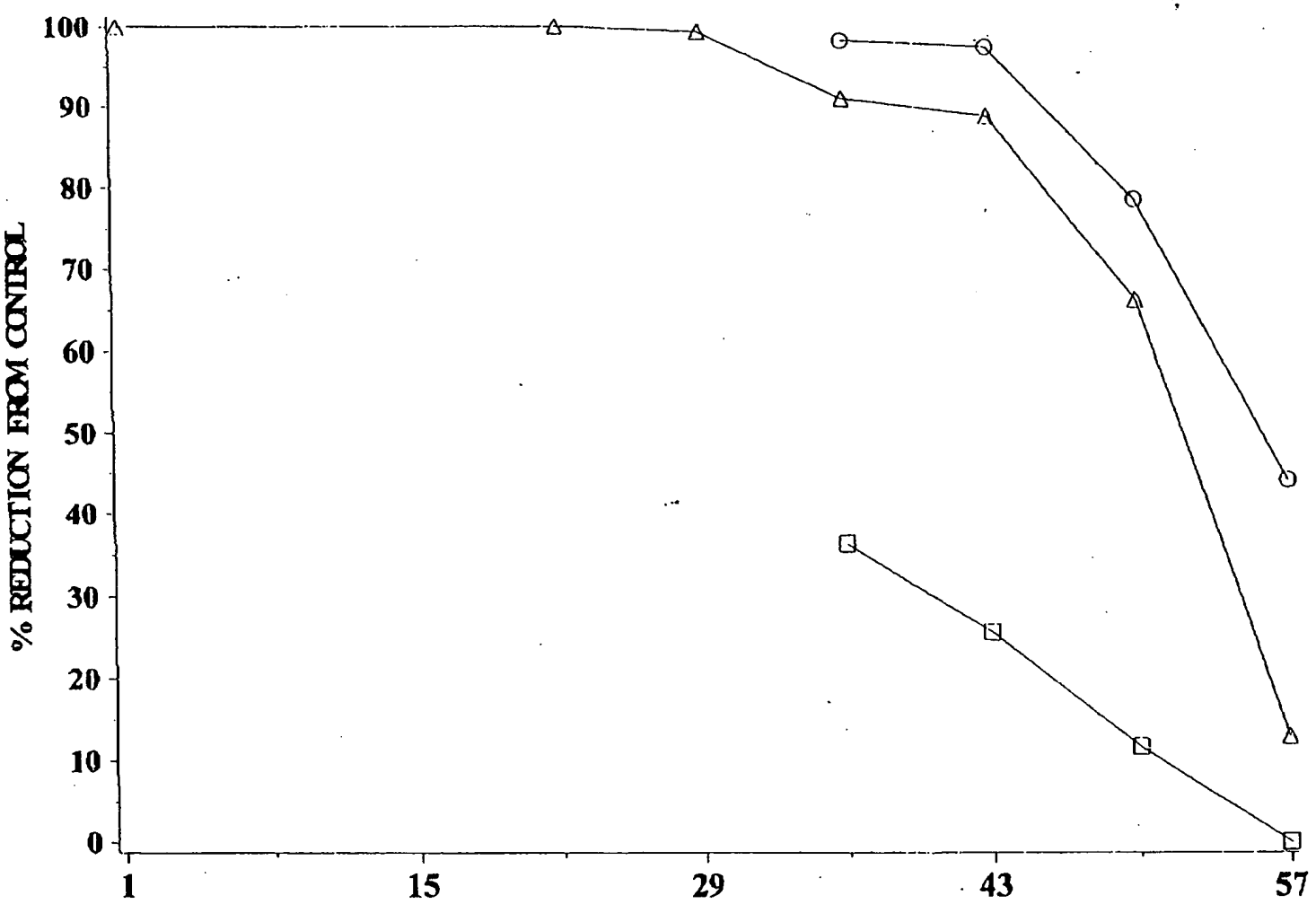

Fig. 2 - % Reduction in Proportion of Adults that Developed by Treatment and Day of Flea Challenge (Eight Cats per Treatment)

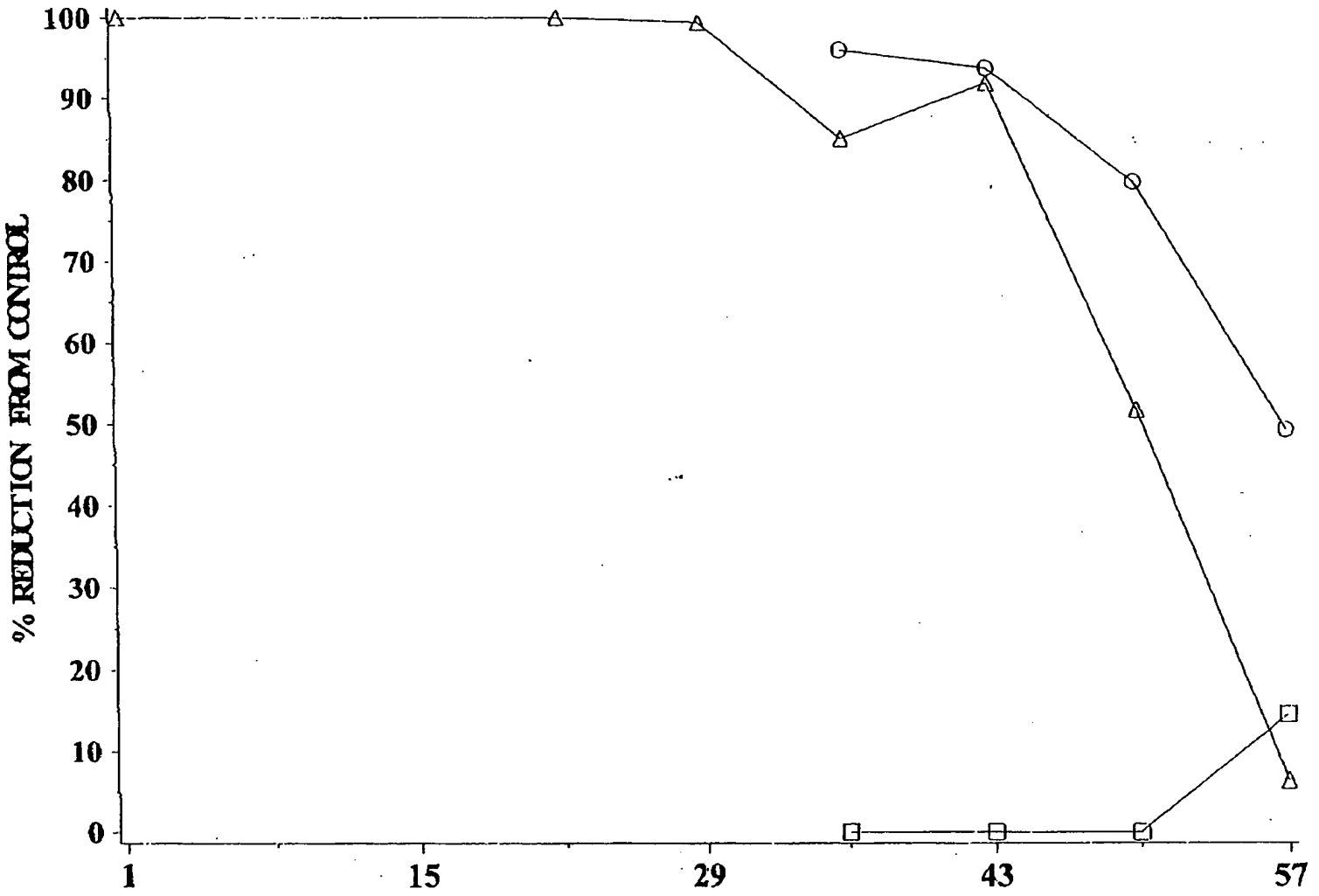

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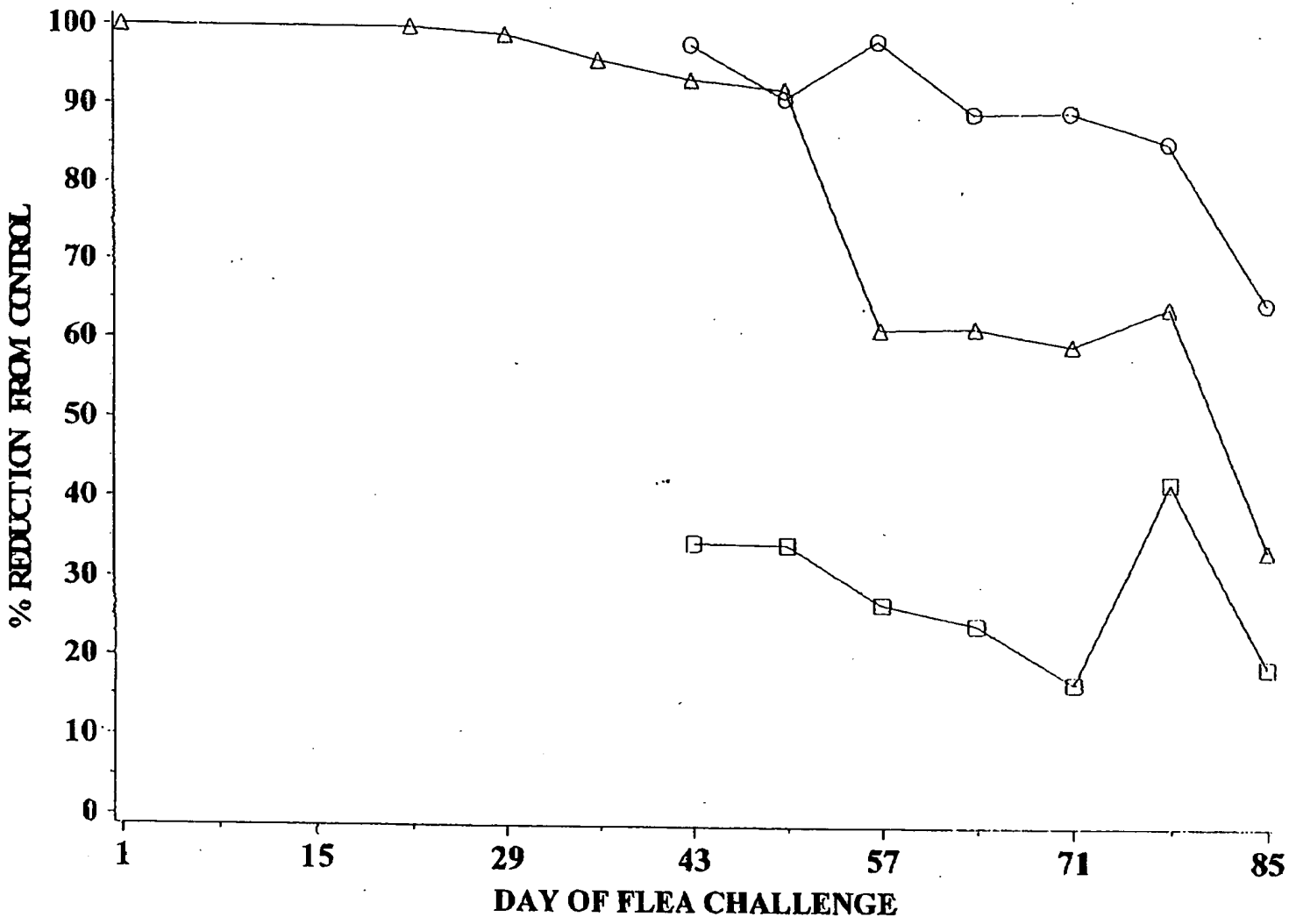
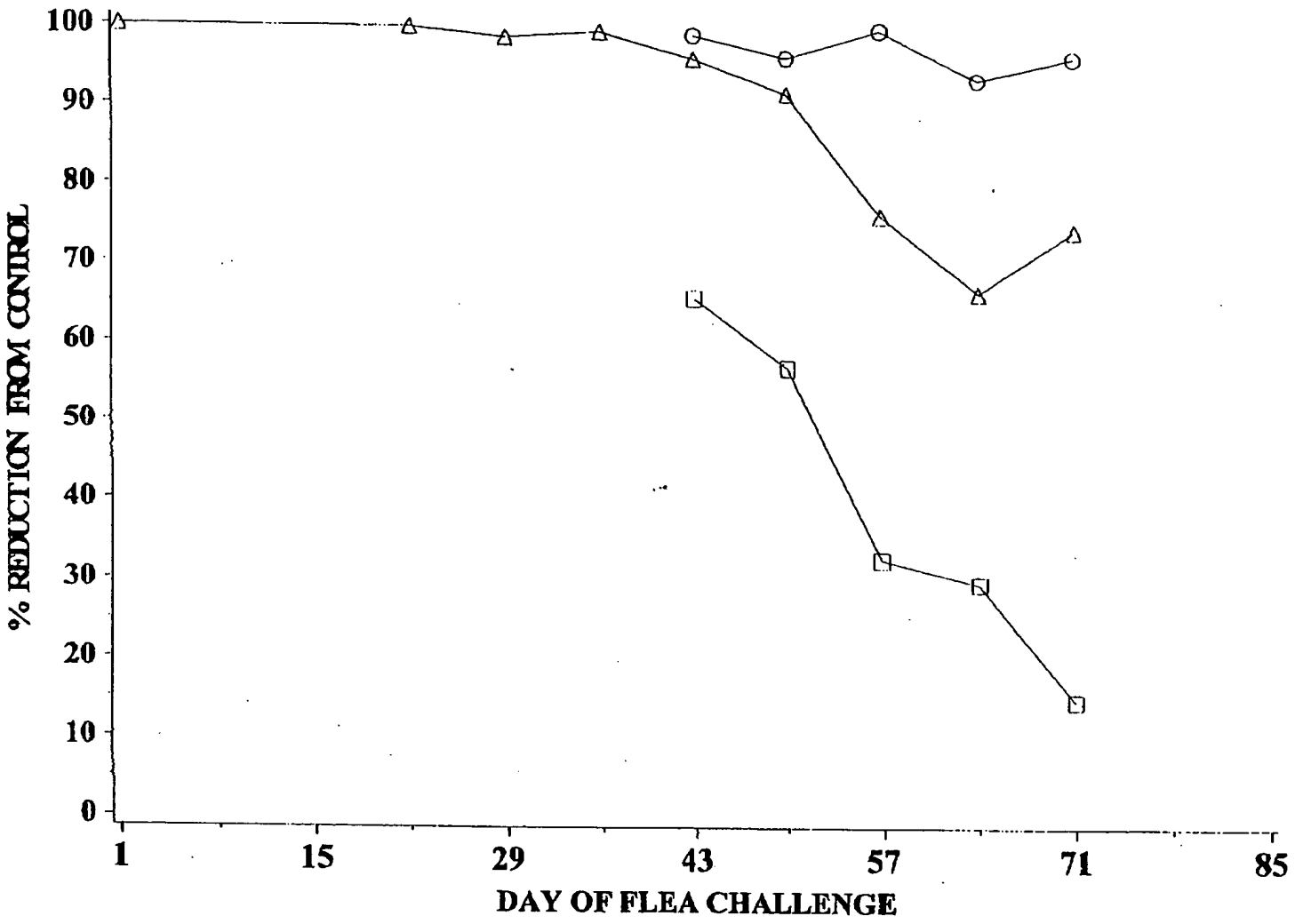
Fig. 3 - % Reduction in Proportion of Larvae that Hatched by Treatment and Day of Flea Challenge (Eight Dogs per Treatment)



SQUARE=FIPRONIL TRIANGLE=METHOPRENE CIRCLE=FIPRONIL+METHOPRENE

Fig. 4 - % Reduction in Proportion of Adults that Developed by Treatment and Day of Flea Challenge (Eight Dogs per Treatment)



SQUARE=FIPRONIL TRIANGLE=METHOPRENE CIRCLE=FIPRONIL+METHOPRENE

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Eur J Drug Metab Pharmacokinet 1997 Jul-Sep;22(3):211-6

Skin distribution of fipronil by microautoradiography following topical administration to the beagle dog.

Cochet P, Birckel P, Bromet-Petit M, Bromet N, Weil A

'Biotec Centre, Orleans, France.

To investigate the localisation of fipronil in dog skin, [14C]-fipronil was topically applied to a male beagle dog (spot-on administration) at the therapeutic dose of 10 mg/kg. By means of autohistoradiography, the radioactivity was precisely detected in the skin and appendages at various intervals after application. Radioactivity was predominantly observed within the stratum corneum, the viable epidermis, and in the pilo-sebaceous units (mainly in the sebaceous glands and epithelial layers). [14C]-fipronil was significantly detected in these structures up to 56 days post-treatment, in the application zone (neck) but also in the lumbar zone, thus indicating the mechanical displacement of fipronil. No radioactivity was detected in either the dermal or the hypodermal layers, confirming the low percutaneous passage of fipronil.

PMID: 9358201, UI: 98023038

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Proc Natl Acad Sci U S A 1996 Nov 12;93(23):12764-7

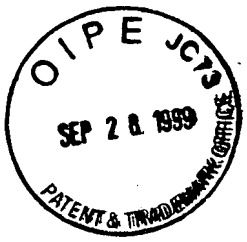
Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity.

Hainzl D, Casida JE

Department of Environmental Science, Policy, and Management, University of California, Berkeley 94720-3112, USA.

Fipronil is an outstanding new insecticide for crop protection with good selectivity between insects and mammals. The insecticidal action involves blocking the lambda-aminobutyric acid-gated chloride channel with much greater sensitivity of this target in insects than in mammals. Fipronil contains a trifluoromethylsulfinyl moiety that is unique among the agrochemicals and therefore presumably important in its outstanding performance. We find that this substituent unexpectedly undergoes a novel and facile photoextrusion reaction on plants upon exposure to sunlight, yielding the corresponding trifluoromethylpyrazole, i.e., the desulfinyl derivative. The persistence of this photoproduct and its high neuroactivity, resulting from blocking the lambda-aminobutyric acid-gated chloride channel, suggest that it may be a significant contributor to the effectiveness of fipronil. In addition, desulfinylfipronil is not a metabolite in mammals, so the safety evaluations must take into account not only the parent compound but also this completely new environmental product.

PMID: 8917493, UI: 97075066



Chem Res Toxicol 1998 Dec;11(12):1529-35

Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct.

Hainzl D, Cole LM, Casida JE

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Fipronil, an N-phenylpyrazole with a trifluoromethylsulfinyl substituent, initiated the second generation of insecticides acting at the gamma-aminobutyric acid (GABA) receptor to block the chloride channel. The first generation includes the polychlorocycloalkanes alpha-endosulfan and lindane. In this study, we examine the mechanisms for selective toxicity of the sulfoxide fipronil and its sulfone metabolite and desulfinyl photoproduct relative to their target site interactions in vitro and ex vivo and the importance in fipronil action of biooxidation to the sulfone. Differences in GABA receptor sensitivity, assayed by displacement of 4'-ethynyl-4-n-[2, 3-3H2]propylbicycloorthobenzoate ([3H]EBOB) from the noncompetitive blocker site, appear to be a major factor in fipronil being much more toxic to the insects (housefly and fruit fly) than to the vertebrates (humans, dogs, mice, chickens, quail, and salmon) examined; in insects, the IC50s range from 3 to 12 nM for fipronil and its sulfone and desulfinyl derivatives, while in vertebrates, the IC50 average values are 1103, 175, and 129 nM for fipronil, fipronil sulfone, and desulfinyl fipronil, respectively. The insect relative to the vertebrate specificity decreases in the following order: fipronil > lindane > desulfinyl fipronil > fipronil sulfone > alpha-endosulfan. Ex vivo inhibition of [3H]EBOB binding in mouse brain is similar for fipronil and its sulfone and desulfinyl derivatives at the LD50 dose, but surprisingly, at higher doses fipronil can be lethal without detectably blocking the [3H]EBOB site. The P450 inhibitor piperonyl butoxide, acting in houseflies, increases the metabolic stability and effectiveness of fipronil and the sulfone but not those of the desulfinyl compound, and in mice it completely blocks the sulfoxide to sulfone conversion without altering the poisoning. Thus, the selective toxicity of fipronil and fipronil-derived residues is due in part to the higher potency of the parent compound at the insect versus the mammalian GABA receptor but is also dependent on the relative rates of conversion to the more persistent and less selective sulfone metabolite and desulfinyl photoproduct.

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Ion channels as targets for insecticides.

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Ion channels are the primary target sites for several classes of natural and synthetic insecticidal compounds. The voltage-sensitive sodium channel is the major target site for DDT and pyrethroids, the veratrum alkaloids, and N-alkylamides. Recently, neurotoxic proteins from arthropod venoms, some of which specifically attack insect sodium channels, have been engineered into baculoviruses to act as biopesticides. The synthetic pyrazolines also primarily affect the sodium channel, although some members of this group target neuronal calcium channels as well. The ryanoids have also found use as insecticides, and these materials induce muscle contracture by irreversible activation of the calcium-release channel of the sarcoplasmic reticulum. The arylheterocycles (e.g. endosulfan and fipronil) are potent convulsants and insecticides that block the GABA-gated chloride channel. In contrast, the avermectins activate both ligand- and voltage-gated chloride channels, which leads to paralysis. At field-use rates, a neurotoxic effect of the ecdysteroid agonist RH-5849 is observed that involves blockage of both muscle and neuronal potassium channels. The future use of ion channels as targets for chemical and genetically engineered insecticides is also discussed.

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